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1 **Functional protein rich extracts from bovine and porcine hearts using acid or alkali**  
2 **solubilisation and isoelectric precipitation.**

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9 Running title: Acid/alkaline bovine and porcine extracts

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23 Abstract

24 Alkali solubilisation (ALS) was compared with acid solubilisation (ACS) for preparation of  
25 protein rich extracts from bovine and porcine hearts. ACS and ALS recovered 51.53 - 55.74%  
26 of the total protein from bovine and porcine hearts. All extracts were rich in myofibrillar  
27 proteins with both treatments resulting in reductions in fat, collagen and cholesterol contents  
28 compared with starting materials. At 0% NaCl, ACS and ALS extracts had good gelling  
29 properties with the ALS gels having lower % cook loss. While treatments did not affect gel  
30 hardness, ACS extracts formed gel networks with higher storage modulus after heating and  
31 cooling. At 2% NaCl gel hardness, % cook loss and storage modulus values increased, with  
32 greater increases occurring for ACS extracts. The results show that ALS and ACS based  
33 processes have potential to produce functional ingredients for processed meat products.

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48 Introduction

49 Slaughterhouses generate a significant volume of meat by-products, many of which have low  
50 economic value. The increasing importance of sustainability along with financial pressures  
51 has led to increasing interest in adding or recovering value from all processing streams in the  
52 meat industry (Toldra *et al.*, 2016, Lynch *et al.*, 2018). During recent decades, quantities of  
53 meat by-products produced from slaughterhouses and meat processors, have significantly  
54 increased, while their consumption has gradually declined. Meat by-products are perceived as  
55 unattractive due to their unaesthetic appearance and to consumer concerns about food related  
56 health risks (Lynch *et al.* 2018, Mullen *et al.* 2017).

57 However, many meat by-products are rich in proteins and if processed correctly can be used  
58 for the production of extracts with desirable functional properties (Chernukha *et al.*, 2015,  
59 Lynch *et al.* 2018). It is well known that myofibrillar proteins are mainly responsible for the  
60 gelling properties of meat products (Chen *et al.* 2015). The production of protein rich extracts  
61 from meat by-products represents a good valorization opportunity for the meat industry, and  
62 at the same time can improve consumer acceptance.

63 Various processes have been used to prepare protein rich extracts from offal meat by-  
64 products. These mainly include surimi type processes and pH shift technology (James and  
65 Mireles DeWitt, 2004). pH shift technology involves acid (ACS) or alkaline (ALS)  
66 solubilisation of proteins, with subsequent precipitation of proteins at their isoelectric point  
67 (Omana *et al.*, 2012).

68 In previous studies, researchers have extracted proteins from a variety of meat related raw  
69 materials, including spent hen and duck meat, mechanically separated turkey and chicken  
70 meat, pork and beef lungs, using either ACS or ALS (Khiari *et al.*, 2014, Wang *et al.* 2013,  
71 Nurkhoeriyati *et al.* 2011, Hrynets *et al.* 2010, Selmane *et al.* 2010).

72 In addition, Mireles DeWitt *et al.* (2002) used an ACS process to produce protein rich extracts  
73 from bovine heart which had excellent heat induced gelation properties. Use of ALS

74 processes has been focused on poultry by-products and little information is available on the  
75 use of this type of pH shift technology in red meat by-products. Therefore, this study will  
76 investigate the potential of ALS as a process to prepare protein rich extracts from bovine and  
77 porcine hearts. Yield and functional properties of ALS extracts will be compared with ACS to  
78 determine if the former offers some advantages.

## 79 Materials and methods

### 80 Preparation of bovine and porcine hearts

81 Fresh bovine (from 18-20 months old mixed cross breed steers) and porcine (from 89-108 kg  
82 Landrace pigs) hearts were obtained from local abattoirs 24 hours *post mortem*. Hearts were  
83 trimmed to remove valves, caps and adipose tissue, cut into cubes and minced through a 5  
84 mm plate. Minced hearts were vacuum packed in polyethylene bags and kept at -20 °C until  
85 use.

### 86 Protein extractability

87 Protein extractability of bovine and porcine hearts at different pH values was determined  
88 using the method of Mireles DeWitt *et al.* (2002) with minor modifications. Minced bovine  
89 and porcine hearts (100 g) were thawed overnight at 4 °C. Minced heart was mixed with  
90 dH<sub>2</sub>O (at 4 °C) using a 1:4 w/w ratio and homogenized at high speed, using a Waring blender  
91 for 30 s × 4. In between each homogenization the slurry was placed into an ice bath for 10  
92 min. The slurry was further diluted to a final minced heart: water ratio of 1:9 (w/w) by  
93 addition of dH<sub>2</sub>O (at 4 °C). pH was adjusted from 2.0 to 11.0, in 0.5 pH increments, with 2N  
94 HCl or 2N NaOH. At each pH point, aliquots were taken and centrifuged at 4000 × g for 20  
95 min at 4°C, for determination of soluble protein.

### 96 Preparation of protein rich extracts using ACS or ALS

97 Proteins were extracted at pH 2.0 or 11.0 using the procedure described above. The acidified  
98 (pH 2.0) or alkali treated (pH 11.0) slurry was centrifuged at 4000 × g for 20 min at 4 °C, and

99 the supernatant was poured through a layer of cheese-cloth prior to isoelectric precipitation of  
100 proteins by adjusting the pH to 5.5. Precipitated protein was collected by centrifuging at 4000  
101  $\times$  g for 20 min at 4 °C and excess water in the pellet was removed by centrifuging at 10000  $\times$   
102 g for 15 min at 4 °C. The pH was adjusted to 7.0 with 5% NaHCO<sub>3</sub> before protein  
103 determination. Protein yield was calculated as follows:

104 % yield = [(weight of protein in supernatant after 1st centrifugation – weight of protein in  
105 supernatant after 2<sup>nd</sup> centrifugation) / (initial weight protein in homogenate)]  $\times$  100

#### 106 Proximate composition analysis

107 Protein (N  $\times$  6.25), moisture, fat and ash content was determined using the AOAC methods  
108 (AOAC 2012). Cholesterol in hearts and protein extracts was determined using an enzymatic  
109 colorimetric method (Boehringer Mannheim / R-Biopharm). Collagen in heart and protein  
110 extracts was determined by a colorimetric method for hydroxyproline in meat (Kolar, 1990).

#### 111 Color properties of protein isolates

112 Objective color values ( $L^*$ ,  $a^*$ ,  $b^*$ ) were obtained with a standardized Minolta colorimeter  
113 (CR-400, Tokyo, Japan). A white standard plate was used to calibrate the colorimeter. The  $L^*$   
114 indicated degree of lightness,  $a^*$  indicated redness and  $b^*$  indicated degree of yellowness.

#### 115 SDS-PAGE

116 Sample preparation was conducted as described from Mireles DeWitt *et al.* (2002). Protein  
117 extracts were 1:10 (w/v) diluted with 5% SDS and homogenised with an Ultra Turrax at high  
118 speed. Samples were placed in a water bath at 80 °C for 60 min. Insoluble material were  
119 removed by centrifugation at 8000  $\times$  g for 20 min at 4 °C and protein in the supernatant was  
120 determined with the biuret method. SDS diluted samples were added to sample buffer [0.25M  
121 Tris-HCl at pH 6.8, 10% glycerol, 7.12 mM 2-mercaptoethanol, 2.5% bromophenol blue  
122 (0.05% w/v)] to a final protein concentration of 3 mg/ml. 5  $\mu$ l of samples was loaded on a 4–  
123 20% ready gel (Bio-Rad Laboratories Inc.). The electrophoretic analysis was performed on a

124 PowerPack Basic electrophoresis apparatus (Bio-Rad Laboratories Inc.) at constant 160 V.  
125 The gel was stained in 0.125% Coomassie blue R-250 and destained in 50:40:10 of  
126 water:methanol:acetic acid solution. A high-molecular weight standard was used for the  
127 estimation of apparent molecular weight retention of protein bands (Bio-Rad Laboratories  
128 Inc.).

#### 129 Heat induced gelation of protein extracts

130 Protein content of freshly made protein extracts was standardised at 8% by adding dH<sub>2</sub>O or  
131 NaCl solution to final salt concentration of 2% (w/w). The protein pastes were allowed to  
132 equilibrate overnight at 4 °C, stuffed into gelation tubes (diameter: 1.8 cm, length: 7.9 cm),  
133 which were coated with a siliconising agent (Sigmacote, Sigma-Aldrich). Before cooking  
134 stuffed tubes were centrifuged at 1500 × g, at 4 °C for 30 s, to eliminate any air bubbles. Then  
135 they were heated to 80 °C, at a rate of 1 °C/min, and held at 80 °C for 5 min before cooling in  
136 an ice bath for 60 min. All samples were stored at 4 °C overnight prior to analysis.

#### 137 Gel hardness

138 The gelled samples were allowed to equilibrate at room temperature for one hour. Gel  
139 hardness was evaluated using a texture analyser (TA-XT2i, Stable Micro Systems Ltd.).  
140 Gelled samples were cut to a height of 10 mm and then compressed at 30% of their original  
141 height, with a constant crosshead speed of 1 mm/s by a 2.5 cm acrylic cylinder. Gel hardness  
142 was determined by the peak force during the compression.

#### 143 Cooking loss

144 Cooking loss was determined by weighing the samples before and after cooking following  
145 removal of free water and it was expressed as follows:

146 Cook loss % = [(weight before cooking – weight after cooking) / weight before cooking] ×  
147 100



148 Dynamic rheological measurements

149 Changes in the storage modulus ( $G'$ ) of the protein extracts (8% (w/w) protein with or  
150 without added NaCl) during heating and cooling were monitored using a control stress  
151 rheometer (HR-2, TA instruments, USA) in oscillatory mode using a hatched plate-plate  
152 geometry of 2.5 cm diameter. Small amplitude deformation (0.5%) strain was applied at an  
153 oscillation frequency of 1 Hz and a 2000  $\mu\text{m}$  gap was used. Samples were heated from 4  $^{\circ}\text{C}$  to  
154 80  $^{\circ}\text{C}$  at a rate of 1  $^{\circ}\text{C}/\text{min}$  and then cooled down from 80  $^{\circ}\text{C}$  to 4  $^{\circ}\text{C}$  at the same rate.

155 Statistical analysis

156 Analysis of variance (ANOVA) was carried out using Minitab® 18 (Minitab Ltd, Coventry,  
157 UK) statistical analysis package. The Tukey method was used to obtain grouping information  
158 on the treatment means. Significances in differences were defined at  $p < 0.05$ . Each  
159 experiment was repeated at least three times.

160 Results and discussion

161 Proximate composition of raw bovine and porcine hearts

162 As can be seen in Table 1, there was no significant differences ( $p > 0.05$ ) between bovine and  
163 porcine hearts in terms of % moisture, % fat, % ash and collagen content. Bovine hearts had  
164 significantly ( $p < 0.05$ ) more protein and less cholesterol than porcine hearts.

165 Protein extractability

166 Bovine and porcine hearts proteins exhibited similar extractability behavior over the pH range  
167 of pH 2.0 to 11.0 (Figure 1), with low protein extractability at pH 5 to 8, and highest protein  
168 extractability at pH 2.0 and 11.0. This is in agreement with Mireles DeWitt *et al.* (2002), who  
169 reported that protein extractability from bovine hearts was highest at pH 2.0 and lowest at pH  
170 5.0 to 6.0 in the pH range of 2.0 to 6.0. Nurkhoeriyati *et al.* (2011) investigated the effect of  
171 pH on protein extractability of spent duck meat and found a highest extractability at pH 2.0  
172 and 11.0, as observed in this study but the pH range for minimum solubility was much

173 narrower, with minimum solubility occurring at pH 5.0 to 6.0. These differences may be due  
174 to species and tissue differences, i.e. skeletal muscle vs cardiac muscle.

175 Based on the protein extractability profile (Figure 1) it was decided to carry out acid and  
176 alkali solubilisation at pH 2.0 and 11.0, respectively, followed by isoelectric precipitation at  
177 pH 5.5.

#### 178 Protein recovery and composition of extracts

179 Proximate composition, yield and color characteristics of protein rich extracts from bovine  
180 and porcine hearts are shown in Table 2. For both bovine and porcine hearts there was no  
181 significant difference ( $p > 0.05$ ) in % protein recovered between the ACS and ALS process.  
182 This is in contrast with the results of Nurkhoeriyati *et al.* (2011), who reported that an ACS  
183 process recovered more protein from spent duck meat than an ALS process. These authors  
184 speculated that the ACS process induced more changes to protein structure resulting in great  
185 protein insolubility on adjustment of the pH at the isoelectric point. These differences may  
186 reflect differences in the starting material used.

187 Extracts prepared from bovine hearts had significantly higher protein content ( $p < 0.05$ ) than  
188 those prepared from porcine heart and within a species there was no significant difference ( $p$   
189  $> 0.05$ ) in % protein of extracts between the ACS and ALS process. All of the extracts  
190 produced had significantly ( $p < 0.05$ ) lower lipid, cholesterol and collagen content than the  
191 starting materials. The initial centrifugation step following acid and alkaline solubilisation  
192 sediments membrane lipids and also removes insoluble collagen (Mireles DeWitt *et al.* 2002).

193 There were no significant differences ( $p > 0.05$ ) in  $L^*$  and  $b^*$  values for any of the extracts.

194 The  $a^*$  values indicate that irrespective of species ALS extracts were redder than ACS  
195 extracts. Using spent duck meat as the starting material Nurkhoeriyati *et al.* (2011) also  
196 reported that an ALS extracts showed greater retention of myoglobin than ACS extracts  
197 which was attributed to greater co-precipitation of myoglobin during isoelectric precipitation.

198 The ALS extracts were visually redder than ACS extracts which may render them more  
199 suitable for use as an ingredient in a sausage type product.

#### 200 SDS PAGE

201 SDS electrophoresis indicates that irrespective of the solubilisation process or species the  
202 protein profile is dominated by the major myofibrillar proteins myosin and actin (Figure 2).  
203 Similar protein profiles were obtained by Mireles DeWitt *et al.* (2002) for acid solubilised  
204 proteins from bovine hearts. The major differences observed in protein profile between ACS  
205 and ALS process the higher intensity of the protein band of 100 kDa in the ALS extracts and  
206 the presence of a protein band with approximate molecular weight 70 kDa in the ACS  
207 extracts. Further studies will be required to determine the identity of these bands.

#### 208 Heat induced gel hardness and cook loss

209 Heat induced gels were prepared from the extracts after standardising the protein content at  
210 8% (Table 3). Addition of NaCl is a key requirement for good gelling properties for  
211 myofibrillar protein rich extracts prepared from bovine hearts using a surimi like processes  
212 (James and Mireles DeWitt, 2004). In this study at 0% added NaCl all extracts formed self-  
213 supporting gels on heating. There was no significant difference ( $p > 0.05$ ) in hardness values  
214 of the gels between process or species. This suggests that ACS and ALS treatment result in  
215 modification of the protein structure which facilitates gel formation in the absence of added  
216 NaCl. The results indicate that these extracts have potential applications as gelling ingredients  
217 in low salt comminuted meat products. When 2% NaCl was added to the extracts gel hardness  
218 increased with a greater increase occurring for the ACS extracts.

219 ALS extracts had significantly ( $p < 0.05$ ) lower % cook loss than ACS extracts (Table 3),  
220 with and without the addition of NaCl, which highlights the potential of ALS extracts for use  
221 as a water binding ingredient in processed meat products. Addition of 2% NaCl increased the  
222 % cook loss for ACS and ALS extracts irrespective of species. This suggests that the presence

223 of NaCl may promote protein-protein interactions, as seen in the increased gel hardness  
224 values, but had an adverse effect on protein-water interactions.

#### 225 Dynamic rheological measurements

226 The storage modulus of ACS and ALS extracts (protein concentration was standardized at  
227 8%) was determined upon heating and cooling. At 0% added NaCl a sigmoidal pattern of  
228 storage modulus during heating was observed for both ACS and ALS treated samples (Figure  
229 3a and 3b). Storage modulus was stable up to 50 °C, where it started decreasing until it  
230 reached a minimum at 56 °C, then it rose steadily up to 80 °C. This pattern implies the  
231 denaturation of myosin molecules, their subsequent aggregation and network formation (Sun  
232 and Holley, 2011). Upon the addition of 2% NaCl storage modulus showed a small decline up  
233 to 60 °C where it started increasing. During cooling storage modulus increased steadily until  
234 the end of the cycle due to the formation of hydrogen bonds (Hrynets *et al.*, 2010), resulting  
235 in a firm gel structure (Ingadottir and Kristinsson, 2010) for all the samples.

236 Storage modulus at 4 °C (after heating and cooling) showed significant variations between  
237 treatments for both species (Table 4). Regardless NaCl addition, ACS treated samples  
238 produced stronger gels than ALS treated, which is directly related to the extent of protein  
239 cross-linking. ACS treated proteins is believed to have more exposed hydrophobic groups on  
240 their surface than ALS treated (Nolsøe and Undeland, 2009) and therefore protein-protein  
241 interactions and cross-linking are promoted. In all samples, addition of 2% NaCl had a  
242 positive effect on storage modulus, with ACS treated samples to exhibit a greater increase  
243 than ALS treated. This comes to accordance with the gel hardness results of this study.

244 Similar results were obtained by Hrynets *et al.* (2010), who reported that at 2.5% NaCl ACS  
245 treated mechanically separated turkey, after heating at 80 °C and cooling at 5°C, showed  
246 higher storage modulus value than ALS treated samples.

247

248

249 Conclusions

250 The ALS process produced protein rich extracts from porcine and bovine hearts with  
251 excellent heat gelling properties and low % fat and cholesterol content. The ALS process has  
252 some advantages over ACS, as the extracts had a redder colour which would be desirable in  
253 many sausage type products. In addition, ALS extracts had lower % cook loss which is of  
254 economic benefit. The ability of ACS and ALS extracts to form gels in the absence of added  
255 NaCl suggests that these wet protein extracts have potential as ingredients in low salt meat  
256 products.

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321 Tables

322 Table 1. Composition of trimmed and minced raw bovine and porcine hearts. Data are  
 323 presented as mean (n=5) ± standard deviation. Means within same row with different  
 324 superscript are significant different (p < 0.05).

|                               | <b>Bovine Heart</b>        | <b>Porcine Heart</b>       |
|-------------------------------|----------------------------|----------------------------|
| <b>Moisture (%)</b>           | 76.71 ± 0.14 <sup>a</sup>  | 76.37 ± 0.96 <sup>a</sup>  |
| <b>Protein (%)</b>            | 19.81 ± 0.48 <sup>a</sup>  | 18.28 ± 0.40 <sup>b</sup>  |
| <b>Fat (%)</b>                | 1.05 ± 0.38 <sup>a</sup>   | 1.54 ± 0.39 <sup>a</sup>   |
| <b>Ash(%)</b>                 | 1.24 ± 0.20 <sup>a</sup>   | 1.52 ± 0.25 <sup>a</sup>   |
| <b>Cholesterol (mg/100 g)</b> | 122.85 ± 3.29 <sup>a</sup> | 130.46 ± 1.15 <sup>b</sup> |
| <b>Collagen (mg/100 g)</b>    | 2.12 ± 0.0 <sup>a</sup>    | 1.74 ± 0.28 <sup>a</sup>   |

325

326 Table 2. Yield, composition and color properties of protein rich extracts from bovine and  
 327 porcine hearts using acid or alkali solubilisation followed by isoelectric precipitation. Data are  
 328 presented as mean (n=3) ± standard deviation. Means within same row with different  
 329 superscript are significant different (p < 0.05).

|                               | <b>Bovine Heart</b>         |                           | <b>Porcine Heart</b>        |                            |
|-------------------------------|-----------------------------|---------------------------|-----------------------------|----------------------------|
|                               | <b>pH 2.0</b>               | <b>pH 11.0</b>            | <b>pH 2.0</b>               | <b>pH 11.0</b>             |
| <b>Yield (%)</b>              | 53.30 ± 4.51 <sup>a</sup>   | 51.53 ± 4.68 <sup>a</sup> | 55.74 ± 3.48 <sup>a</sup>   | 52.01 ± 2.48 <sup>a</sup>  |
| <b>Protein (%)</b>            | 12.27 ± 0.48 <sup>a,b</sup> | 14.33 ± 0.89 <sup>a</sup> | 10.68 ± 0.76 <sup>b</sup>   | 11.31 ± 0.73 <sup>b</sup>  |
| <b>Moisture (%)</b>           | 81.60 ± 2.43 <sup>b</sup>   | 81.92 ± 0.69 <sup>b</sup> | 87.17 ± 0.75 <sup>a</sup>   | 85.82 ± 0.05 <sup>a</sup>  |
| <b>Fat (%)</b>                | 0.28 ± 0.042 <sup>b</sup>   | 0.29 ± 0.04 <sup>b</sup>  | 0.36 ± 0.02 <sup>a</sup>    | 0.37 ± 0.06 <sup>b</sup>   |
| <b>Ash (%)</b>                | 0.39 ± 0.039 <sup>b</sup>   | 0.65 ± 0.09 <sup>a</sup>  | 0.39 ± 0.07 <sup>b</sup>    | 0.55 ± 0.13 <sup>a,b</sup> |
| <b>Cholesterol (mg/100 g)</b> | 52.15 ± 1.6 <sup>c</sup>    | 73.98 ± 6.56 <sup>b</sup> | 82.06 ± 3.95 <sup>a,b</sup> | 92.14 ± 10.50 <sup>a</sup> |
| <b>Collagen (mg/100 g)</b>    | 0.45 ± 0.06 <sup>a</sup>    | 0.42 ± 0.04 <sup>a</sup>  | 0.38 ± 0.03 <sup>a</sup>    | 0.20 ± 0.01 <sup>b</sup>   |
| <b>Color properties</b>       | <i>L</i> *                  | 38.99 ± 2.40 <sup>a</sup> | 38.93 ± 1.13 <sup>a</sup>   | 34.74 ± 1.56 <sup>a</sup>  |
|                               | <i>a</i> *                  | -0.13 ± 0.09 <sup>b</sup> | 2.75 ± 0.38 <sup>a</sup>    | 2.40 ± 0.59 <sup>a</sup>   |
|                               | <i>b</i> *                  | 7.49 ± 0.76 <sup>a</sup>  | 7.25 ± 1.41 <sup>a</sup>    | 7.17 ± 0.46 <sup>a</sup>   |

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331 Table 3. Hardness (g) and cook loss (%) of protein rich extracts from bovine and porcine  
 332 hearts using acid or alkaline solubilisation isoelectric precipitation, after heating (4 °C to 80  
 333 °C) and cooling (4 °C). Samples protein content was adjusted to 8 % (w/w) and 0% or 2%  
 334 NaCl was added. Data are presented as mean (n=4) ± standard deviation. Means within same  
 335 row with different superscript are significant different (p < 0.05).



|                     | ACS Bovine              |                         | ALS Bovine               |                          | ACS Porcine               |                         | ALS Porcine              |                         |
|---------------------|-------------------------|-------------------------|--------------------------|--------------------------|---------------------------|-------------------------|--------------------------|-------------------------|
|                     | 0% Salt                 | 2% Salt                 | 0% Salt                  | 2% Salt                  | 0% Salt                   | 2% Salt                 | 0% Salt                  | 2% Salt                 |
| <b>Hardness (g)</b> | 123.3±0.75 <sup>d</sup> | 184.8±2.42 <sup>a</sup> | 119.54±0.73 <sup>d</sup> | 138.7±4.28 <sup>c</sup>  | 127.7±0.75 <sup>c,d</sup> | 184.5±2.48 <sup>a</sup> | 118.47±4.02 <sup>d</sup> | 148.6±4.47 <sup>b</sup> |
| <b>% Cook loss</b>  | 25.9±0.18 <sup>d</sup>  | 31.17±0.27 <sup>a</sup> | 20.8±0.4 <sup>e</sup>    | 27.4±1.98 <sup>c,d</sup> | 29.6±0.11 <sup>b,c</sup>  | 33.6±0.19 <sup>a</sup>  | 17.8±1.1 <sup>f</sup>    | 27.17±0.58 <sup>d</sup> |

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337 Table 4. Storage modulus of protein rich extracts from bovine and porcine hearts, using acid  
338 or alkali solubilisation followed by isoelectric precipitation, at 4 °C after heating from 4 °C to  
339 80 °C followed by cooling from 80 °C to 4 °C at 1 °C/min. Samples protein content was  
340 adjusted to 8 % (w/w) and 0% or 2% NaCl was added. Data are presented as mean (n=3) ±  
341 standard deviation. Means within same row with different superscript are significant different  
342 ( $p < 0.05$ ).

|                | ACS Bovine                   | ALS Bovine                   | ACS Porcine                  | ALS Porcine                  |
|----------------|------------------------------|------------------------------|------------------------------|------------------------------|
| <b>0% NaCl</b> | 26671.1 ± 305.5 <sup>a</sup> | 13542.3 ± 266.6 <sup>b</sup> | 25063.5 ± 785.6 <sup>a</sup> | 17134.4 ± 493.0 <sup>c</sup> |
| <b>2% NaCl</b> | 32499.9 ± 335.7 <sup>a</sup> | 17712.9 ± 403.2 <sup>b</sup> | 30675.3 ± 791.8 <sup>a</sup> | 26100.2 ± 916.1 <sup>c</sup> |

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355 Figures legends

356 Figure 1. The extractability (mg/ml) profile of proteins recovered from bovine (○) and porcine  
357 (□) hearts at the pH range from 2.0 to 11.0.

358 Figure 2. SDS-PAGE profile of protein rich extracts from bovine and porcine hearts using  
359 acid or alkali solubilisation followed by isoelectric precipitation. (Lane 1: high-molecular-  
360 weight standard, lane 2: ALS extract from porcine heart, lane3: ACS extract from porcine  
361 heart, lane 4: ALS extract from bovine heart, lane 5: ACS extract from bovine heart).

362 Figure 3. Rheograms of protein rich pellets from bovine (a) and porcine (b) hearts using acid  
363 or alkaline solubilisation, isoelectric precipitation with 0% or 2% NaCl addition. The  
364 rheograms show storage modulus during heating from 4 °C to 80 °C followed by cooling from  
365 80 °C to 4 °C at 1 °C/min. Samples protein content was adjusted to 8 % (w/w) and 0% or 2%  
366 NaCl was added.

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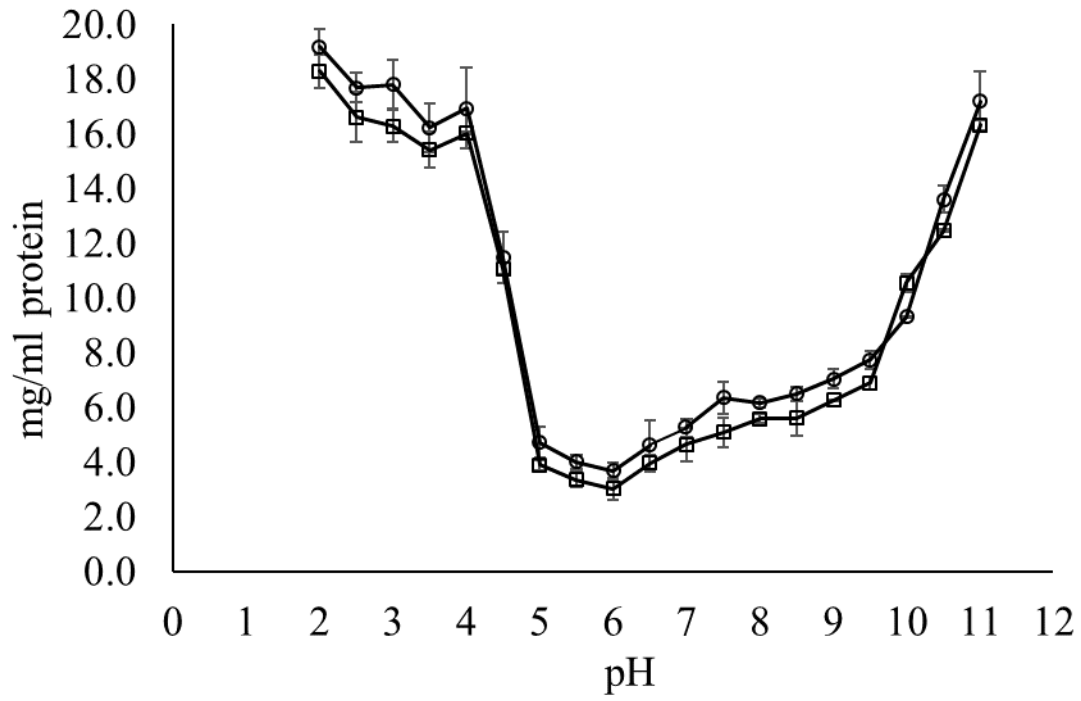
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378 Figures

379 Fig. 1



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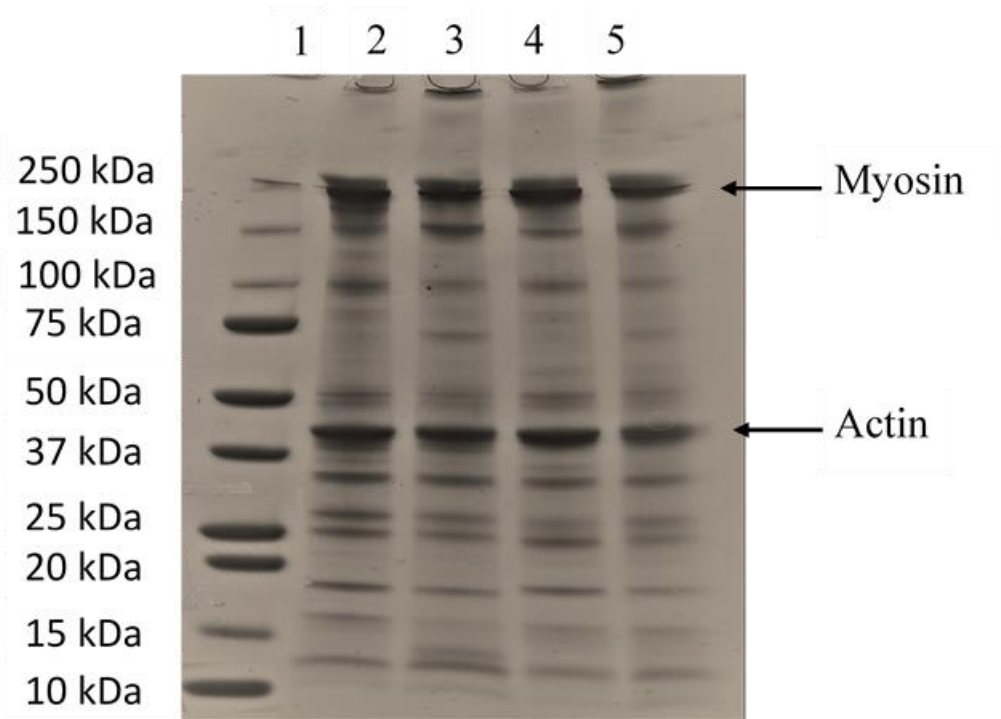
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391 Fig. 2



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